UNIVERSITY OF PITTSBURGH
GRADUATE SCHOOL OF PUBLIC HEALTH
PITTSBURGH, PENNSYLVANIA 15213

May 31, 1967

Grants and Research Contracts
Code SC
Office of Space Sciences
National Aeronautics and Space Administration
Washington, D.C. 20546

Dear Sir:

This is the thirteenth quarterly progress report submitted in accordance with the requirements of NASA Contract NASr-169 covering the period from 1 January 1967 through 31 March 1967.

Automatic Microscope: On January 31, 1967 we were notified by the Perkin-Elmer Company that Mr. R.D. McLaughlin, the Project Manager and Mr. Martin Yellin, his associate, had been transferred to another project due to company manpower requirements. In addition, the further assembly of the microscope had been stopped due to lack of funds. Investigations carried on through the month of February culminated in a visit to the company on March 3. At that time, all of the components of the automatic microscope were in hand except the rotating platen. Dr. Sternglass, Mr. Ranshaw and the undersigned felt that the stage of development of the microscope was such that it would be most efficient to provide further funds and time for its completion by the Perkin-Elmer Company. Accordingly, negotiations were pursued and completed with NASA and the Perkin-Elmer Company to amend the sub-contract by adding $12,208 in additional funds and extending the period of performance from March 8,1967 to June 30, 1967.

In view of the progressive delays in the delivery date of the automatic microscope, it was deemed advisable to have the Perkin-Elmer Company deliver the breadboard scanner utilized under our sub-contract No. 1 for the automatic microscope feasibility study. It was felt that preliminary data could be procured concerning possible needs for refinement in cytogenetic technique with this relatively crude apparatus. Accordingly, the breadboard scanner was delivered on the 24th of March 1967. Its checkout was then initiated.

Computer Interface: The design plan for preparation of the PDP-7 computer to function as the control system for the automatic microscope and the flying spot scanner was reviewed in detail in our twelfth quarterly progress report. The first three sections below indicate the additional progress toward the realization of these plans. The fourth and fifth sections below outline the functions to be performed on the automatic microscope and the programming (software) which will be required to carry out these operations.

I. PDP-7 Real-Time Connection Expansion

All sections of the expanded interface have been connected into the PDP-7 and are functioning properly. That is, by issuing the appropriate IOT commands by means of PDP-7 test programs, the expected control pulses and levels are generated by the interface logic. A few minor writing errors, as well as design errors, were detected and corrected.

II. Interface to the Automatic Microscope

The interface is still in the preliminary design stage. Further work had been delayed pending receipt of up-to-date information from Perkin-Elmer. This data has been received and the design will proceed shortly.

The connectors required for coupling the PDP-7 to the microscope have been ordered and delivered.

The Analog-to-Digital converter has been connected into the system and performs satisfactorily in a digital manner. The necessary analog portions are as yet untried.

III. Flying Spot Sub-System

All of the logic portions of the sub-system have been checked and are functioning properly. The Digital to Analog conversion ladder networks have not as yet been installed.

The Celco deflection and focus coil drivers were tested and found to be in apparent working condition. However, there have been no quantitative evaluations made.

A motor-actuated timing switch has been chosen as the primary timing element of the power sequencer. Other features of the sequencer are as yet unspecified.

IV. Automatic Microscope Functions

The microscope, designed and built by the Perkin-Elmer Company, is specially designed. It is so constructed as to permit full automatic operation through the use of a control computer. Figure 1 indicates the major features of the device.

The primary goal of the system is to rapidly screen every 50 micron area of a microscope slide for the presence of a metaphase spread of human blood cells. Under a sub-contract, Perkin-Elmer devised a means of doing this through the use of spatial Fourier Transform processing. Details of this work may be found in their Engineering Report, Number 8060, dated July 23, 1965. Information on the Fourier Transform technique may be found in the proceedings of the Symposium on Optical and Electrooptical Information Processing, Chapter 4, page 59. The application of this technique in the microscope is realized in the "Laser System" by using a Hellium-Neon Laser to form a 50 micron spot of coherent light on a microscope slide, which in turn is moved via the rotating platen. Optics, placed above the slide, form the two-dimensional Fourier Transform of the illuminated area. This transform is then optically broken down into high-and-low spatial frequency components. The intensity of these components is measured by means of photomultiplier tubes. Report 8060 describes a method of processing of these intensity measurements to detect a possible metaphase spread.

During this search phase, the platen is in a free-wheeling state, rotating at about 20 RPM. This rate is sufficient to observe 3 million 50 micron fields per hour. Once a suspected area has been detected, the platen is stopped. In order to further process the area, it is now necessary to re-position the platen so that the region in question lies under the Visual System. This is accomplished by employing a closed-loop digital servo system, where the PDP-7 computer closes the loop. Feedback is provided by means of digital shaft encoders, a 17-bit unit indicating

platen angle, and a 12-bit one to indicate radial motion. By reading these encoders, the computer can calculate the slide number (there are six on the platen), the position of the cell on the slide, and the necessary changes to the radius and angle required to move the cell under the Visual System. Computer control for doing this is provided by using incremental stepping motors and suitable gearing.

Having placed the cell under the Visual System, the computer now enters a focusing phase. The loop in this case consists of the Flying-Spot-Scanner Sub-system to gather data, and another stepping motor arranged to move the objective lens of the visual microscope in 0.1 micron steps. Again, a 12-bit encoder provides feedback to the computer about this motion.

Further analysis of the cell may now be made by using the Flying-Spot-Scanner Sub-system to scan the area. This task may in turn be given to a larger computer, such as a PDP-10. After this analysis, the slide may be photographed, a data-block recorded on film via the Scanner CRT, and the search-mode resumed.

In addition to the above-mentioned functions, the computer also has direct control over various shutters for the photomultipliers and light sources, and the 35 mm. camera.

V. Programming

The programming tasks may be divided into four main areas: Searching, Re-positioning, Focusing, and General Services.

Searching:

This involves monitoring of the high-and-low frequency photomultipliers for a suspected metaphase cell, and advancement of the radial system. Perkin-Elmer has specified a simple threshold and ratio comparison of the two Fourier signals. This may be done by means of a program, or through the use of some analog circuitry. There are decided advantages to using analog methodology and this probably will be provided. Since the test is fairly simple, no difficulty is anticipated in the design. Initially, however, it will be possible to do the comparison by software. With an A/D conversion time of 9 microseconds, it is estimated that the comparison can be done in 20 microseconds, using the parallel operation capabilities of the converter. At this rate, some twenty samples are taken of every 50 micron area passed over. This should be adequate resolution.

Having detected a cell, the program proceeds to the Re-positioning phase. This consists of reading the platen shaft encoder and saving the value. This indicates the relative position of the slide and the cell on the slide. The radial encoder is also saved, and the free-wheeling of the platen is stopped.

Re-positioning:

In order to re-position the cell from the Laser System to the Visual System, it is necessary to compute a correction to the relative angular position of the platen. Figure 2b illustrates the need to perform this calculation, rather than employ a constant correction regardless of position. As successive scans of the slide are made, the radial location of the platen's center is changed relative to the location of the microscope positions. In the figure, the solid lines represent the situation at nearly the greatest possible separation of platen center and Laser System. The dotted lines correspond to the minimal distance. As can be seen, the correction angles, θ_1 and θ_2, vary.

If it is assumed that the radial component is the perpendicular bisector of the line drawn between the centers of the two optical chains, then it would only be necessary to change the relative angular position. However, due to the tight dimensional requirements (0.4×10^{-3} inches), it is somewhat unrealistic to assume this. Figure 2b shows the general case, in which this condition does not hold. Here there are three parameters to be determined: d_1, the $\perp$ distance from the Laser System to the radial vector; d_2, the $\perp$ distance from the Visual System to the radial vector; d_3, the separation between the projection of d_1 and d_2 along the radial vector. It is necessary to calculate λ_1, λ_2 and ΔR to correct the angular position and radial position, respectively. Since the distance from the platen center to the cell will not change, it is the basic measure in the calculation, and can be obtained by using the Pythagorean theorem. These corrections are given in figure 2b. It should be noted, that if $d_1 = d_2$, and $d_3 = 0$, these corrections reduce to $\Delta R = 0$ and $\lambda_1 = \lambda_2 = \text{ARCSIN} \frac{2D}{\sqrt{4R^2 + D^2}}$. If the angular correction is determined from figure 5a, the same results are obtained.

All measurements are in terms of the corresponding encoder resolution.

In order to perform these calculations with the required degree of precision, it will be necessary to utilize double-precision arithmetic, wherein each number occupies two words of PDP-7 memory. DEC has provided a subroutine package for doing this. However, it will be necessary to write the Square-root and Arcsine subroutines. This should require no more than two weeks.

Focus:

Having moved the cell into position under the Visual System, there remains the task of bringing the image into focus. Although the problem is totally unsolved, so far as we know, there do exist some ideas as to course of action. Two possible methods will be discussed. The first is based on the average width of objects, and the second on optical density histograms.

The width measurement is similar to the focus technique outlined by Perkin-Elmer in Report No. 8111, which utilizes video pulse-width measurements. Using the equipment so far described, the procedure would be to scan a few lines by means of the computer, then pick an optical density as a threshold value, and measure the width (number of consecutive points) of areas which exceed this value. The basic precept here is that as an object is racked out of focus, the apparent size increases due to the "fuzzing" of the edges. Having determined the distribution of widths, the focus setting is changed and the process repeated. As long as an "improvement" of focus is detected, this process continues. When the image appears to be degraded, the focus setting is reversed to the optimum point.

The alternate focus method is based on the fact that an out-of-focus image has a lower contrast than one which is in sharp focus. Contrast may be defined as the range of optical densities. By computing optical density histograms and applying statistical comparisons between successive histograms, it should be possible to obtain a measure of focus, and arrive at the optimal degree of focus as above. What these statistical comparisons would be remain to be determined.

It may turn out that a combination of these two methods will be needed for best performances.

General Services:

The General Services programming is comprised of routines to scan the cell with varying specifications, calculation of optical density from the Data and Reference Photomultiplier signals, displaying alphanumeric data on the CRT for recording on film, communication with the PDP-10, and similar tasks.

It is estimated that within three to six months the software should be in a usable, though not perfect, state.

Respectfully submitted,

Niel Wald

Niel Wald, M.D.
Professor of Radiation Health

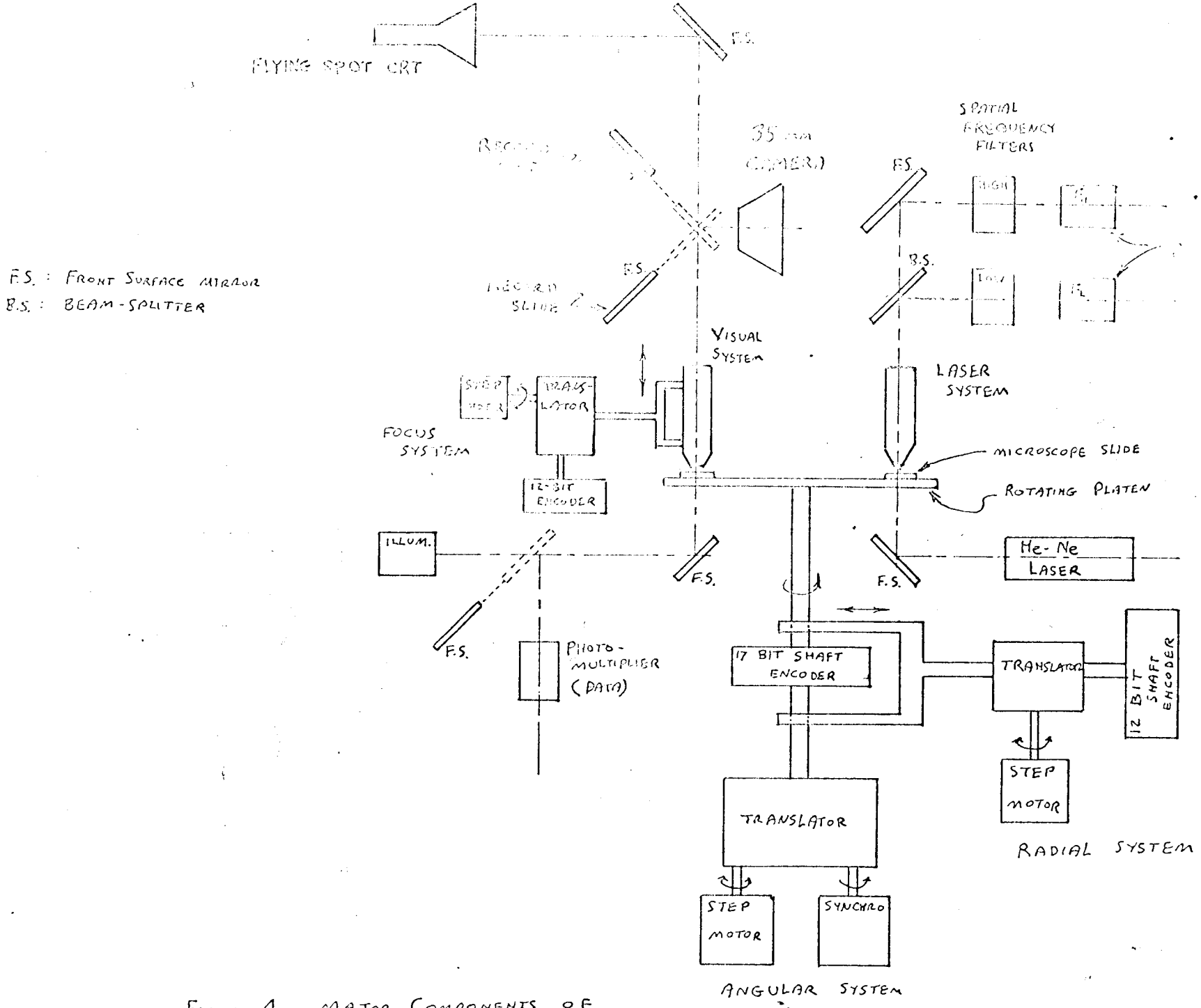
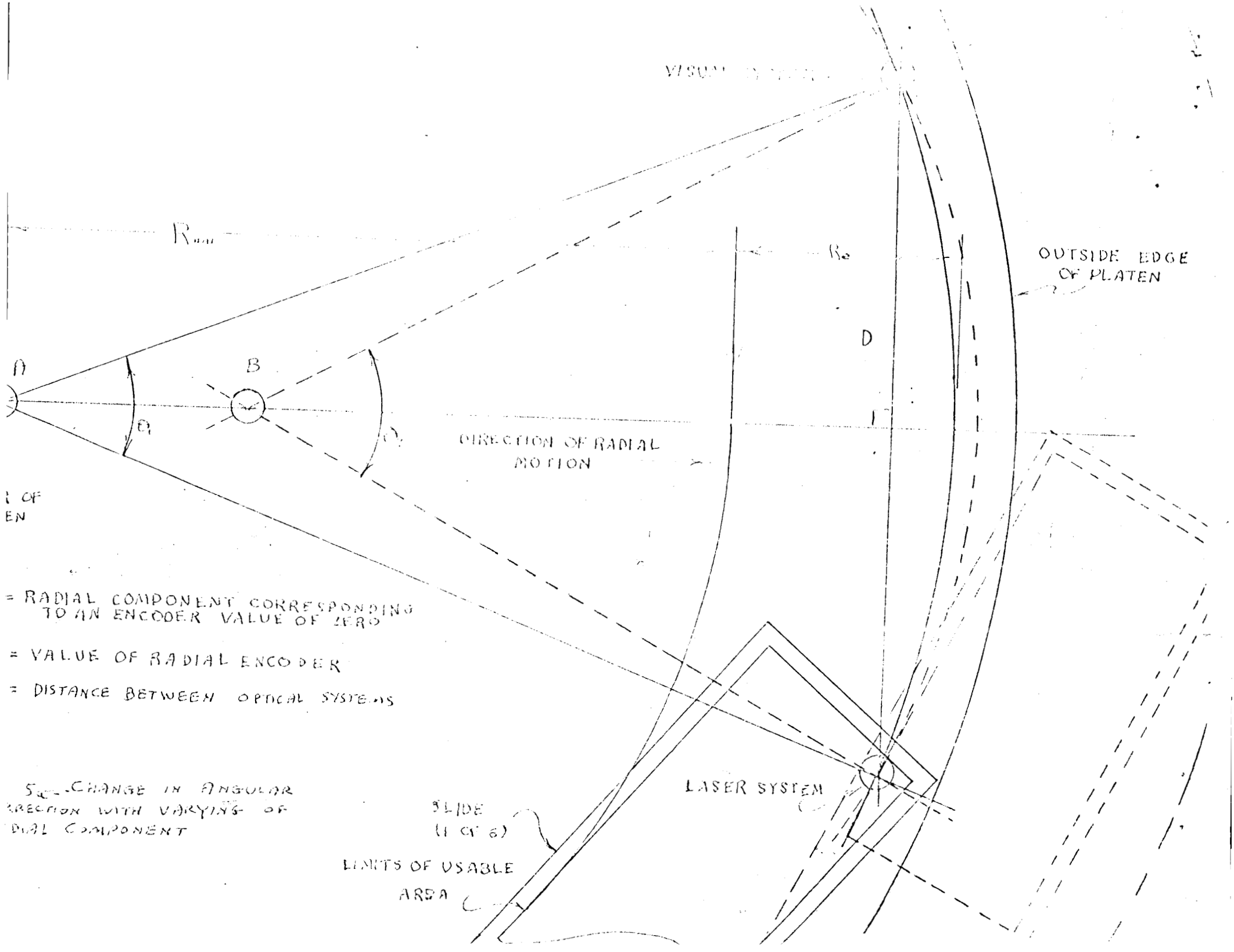


FIGURE 4 - MAJOR COMPONENTS OF THE AUTOMATIC MICROSCOPE.



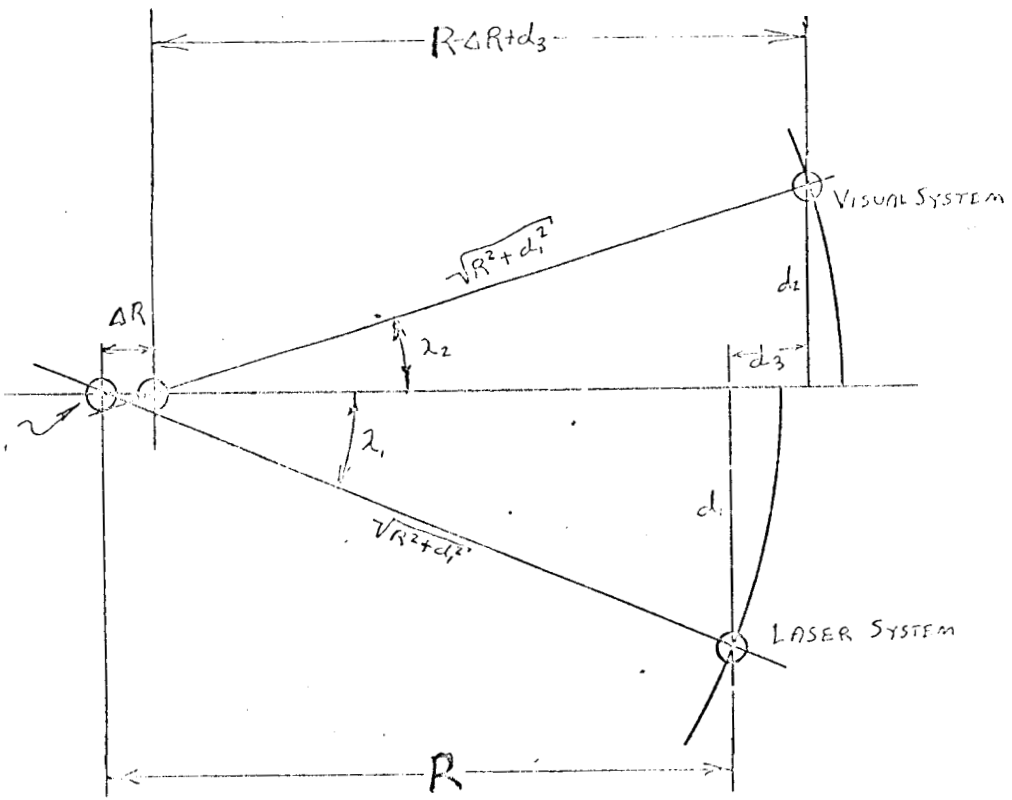
OF
EN

= RADIAL COMPONENT CORRESPONDING TO AN ENCODER VALUE OF ZERO

= VALUE OF RADIAL ENCODER

= DISTANCE BETWEEN OPTICAL SYSTEMS

S... CHANGE IN ANGULAR
RECTION WITH VARYING OF
DIAL COMPONENT



CORRECTIONS NEEDED TO R AND Θ TO RE-POSITION AN AREA FROM THE LASER SYSTEM TO THE VISUAL SYSTEM:

$$\Delta R = R + d_3 - \sqrt{R^2 + d_1^2 - d_2^2}$$

$$\lambda_1 = \text{ARCSIN} \frac{d_1}{\sqrt{R^2 + d_1^2}}$$

$$\lambda_2 = \text{ARCSIN} \frac{d_2}{\sqrt{R^2 + d_1^2}}$$

d_1, d_2, AND d_3 MUST BE DETERMINED. ALL MEASUREMENTS SHOULD BE IN TERMS OF THEIR RESPECTIVE ENCODER RESOLUTIONS.

FIGURE 5.2 - THE GEOMETRIC REPOSITIONING PROBLEM